NEWCASTLE DISEASE

A STUDY OF THE CARRIER PROBLEM

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NEWCASTLE disease was first discovered in Canada in February 1948. Almost simultaneously, but independently, Walker (1) and Crawley and Glover (2) isolated the specific virus from specimens received from the same source. Since then, eighteen outbreaks have been found. These were distributed as follows: Nova Scotia 1, Quebec, 1, Ontario 15, Saskatchewan 1.

The majority of these outbreaks occurred in separate localities having no apparent contact with flocks where the disease was known to have existed. It seemed likely therefore that the infective agent had been transferred through the medium of a carrier such as the egg.

Several authors have given consideration to this possibility. Beach (3) isolated the virus from the ovarian tissue of a hen. Jungherr (4) and Van Roekel (5) from fresh eggs, and DeLay (6) from the yolk sac of 4-day old chicks, chick embryos and infertile eggs obtained from parent stock known to be affected with Newcastle disease. Circumstantial evidence also suggests the egg may be important in the transmission of Newcastle disease virus. For example, chicks obtained from a commercial hatchery having no contact with mature birds and coming from eggs that were believed to be the products of healthy hens, developed the infection when approximately four days old. The infective agent must therefore have passed from egg to chick through the medium of the egg or the eggs must have been contaminated on the surface. It was felt that a study should be commenced having as its objective gathering data upon the potential carrier problem.

METHODS

Twelve adult White Leghorn hens and two roosters negative to the haemagglutination-inhibition (HI) test were isolated in a clean pen. They were kept under observation for 14 days during which time the daily egg yield of the flock averaged 8. They were again tested serologically to insure freedom from Newcastle disease.

Embryo tissue containing a virulent strain of virus (Watford) was suspended in normal saline solution in a dilution of 10^{-3} and 0.5 ml. was inoculated intratracheally into each member of the flock; also, 6 chickens of approximately 6 weeks of age, which were kept in a separate unit were inoculated similarly.

Throat swabs and fresh faecal material were collected from the birds weekly and examined for the presence of virus. These materials were diluted in sterile nutrient broth and allowed to incubate for one hour at room temperature, with occasional shaking. The supernate was drawn off and penicillin and streptomycin added to provide 600 units and 200 micrograms

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respectively per milliliter. The supernate from the fecal material was divided into two lots; one lot was passed through a Seitz filter. The various supernates were tested for the presence of virus by the inoculation of 0.2 ml. into the allantoic sac of nine or ten day embryonating eggs.

The presence of the virus was indicated by death of the embryo within three days—usually in 36 to 48 hours. These showed extreme congestion with haemorrhages involving the brain and muscles. The egg fluids were tested for agglutinative activity for chicken red cells, and the identity of the virus in positive fluids confirmed by haemagglutination-inhibition tests (HI) with specific antisera for the Newcastle disease virus.

Bleedings taken weekly from all birds, were tested for the presence of antibody by the haemagglutination-inhibition test with the strain of N.D.V. virus used in inoculation.

Eggs were collected during the 30 days following inoculation and twice weekly thereafter for a twelve-month period. Two or three fresh eggs from each lot were treated with alcohol to reduce surface contamination as much as possible, opened carefully and the contents placed in a Waring blendor. After sufficient agitation, the material was placed in the refrigerator and allowed to settle for two hours. The supernate was tested for the presence of virus by the inoculation of embryonating eggs.

The remaining eggs were placed in the incubator and candled on the seventh and seventeenth day. Those which were non-fertile of which contained dead embryos were removed. Pools were made of the infertile eggs and of the dead embryo tissue. These were homogenized in the Waring blendor and tested for virus in the same manner as the fresh eggs.

The chicks that hatched were placed in an electric brooder and observed daily. Groups of young healthy chicks were placed in the same brooder and also watched carefully for the development of symptoms of Newcastle disease.

RESULTS

The Role of the Egg in the Transmission of Virus. — Six days after inoculation with Newcastle disease virus, symptoms including listlessness, anorexia and mild respiratory difficulty, appeared in adult birds. Egg production ceased and except for a few soft-shelled eggs, did not commence for twelve days; seventy days elapsed before the average production returned to normal. Symptoms also appeared in young chicks about the same time but these were more severe, one bird dying on the fifth day and another on the eleventh day of infection. Virus was recovered from each. Virus was also isolated from throat swabs from both group birds on the seventh, fourteenth and twenty-first days after inoculation. By the twenty-ninth day the virus had disappeared and was not obtained from throat swabs at any later time. Antibodies for Newcastle disease virus had developed in the sera of all birds by the fourteenth day and remained at a high level over the period of observation.

During the period when virus was being regularly demonstrated in the throat swabs, faecal material consistently failed to show the presence of virus; neither was virus ever found in fresh eggs collected from the adult birds during the same period nor subsequently. No virus was detected in the infertile eggs or in the tissues of embryos that died during incubation. On no occasion did Newcastle disease develop either in the young chicks that hatched from the remaining eggs or in young healthy chicks placed in contact with them. Antibodies for Newcastle disease virus could be demonstrated for a short period in sera of certain of the former group, but none was detected in sera of the contact chicks. The transient presence of antibodies in the chicks hatched from eggs of virus-infected hens was apparently due to transfer of antibodies, not to transmission of the infective agent to the egg.

This experiment therefore gave no evidence that Newcastle disease virus is transmitted to the egg by a hen with an acute form of this infection. Furthermore there was no indication that faecal droppings might be implicated as a source of disssemination of the virus, through contamination of the surface of the egg.

The Role of Contact in the Transmission of Virus. — Six months after the White Leghorn flock had first become infected there were added six healthy Barred Rock hens which were serologically negative to the HI test The group was kept under observation for another six months during which time no symptoms developed in the contact birds. Although blood taken from the White Leghorns continued to react to the HI test, demonstrating the continual presence of neutralizing antibodies, no antibodies were found in sera of the additions. Eggs from these birds also proved to be free of virus. Although the previously inoculated hens may still have been harbouring virus, as is suggested by the persistence of high antibody titers, it was not apparently transmissible to other hens housed with them.

At the time the additional hens were added, a reacting rooster was removed and placed in an isolation unit. Six healthy Barred Rock hens negative to HI test were then placed in contact. All went well for five weeks then suddenly a mild respiratory infection appeared in the hens, egg production ceased and one hen presented symptoms of nerve involvement. The infection was transient and passed quickly, egg production being resumed in approximately two weeks. Within a few days antibodies were present in the blood of all hens and in the eggs which were laid. Repeated attempts to isolate the virus from the hens and their eggs failed. Because the group was in strict isolation and since no experiments, in which active virus was employed, were in progress at the time, it is felt that the hens were infected by the rooster.

SUMMARY

1. A flock of twelve mature birds and two roosters experimentally exposed to the virus of Newcastle disease exhibited a high morbidity with mild symptoms of the disease; they recovered without mortality. Six young

chicks similarly exposed as controls developed severe symptoms of infection which resulted in two deaths.

2. The virus of Newcastle disease was found to persist in the respiratory tract of infected birds for at least twenty-one days, after which time it could not be isolated.

3. The virus was not demonstrated in faecal material at any time.

4. Eggs laid during the post-infection period, or chicks hatched from the eggs failed to reveal the presence of the virus.

5. Normal adult hens placed in contact with a flock infected six months previously failed to develop clinical disease or show serological evidence of its presence.

6. Normal adult hens placed in contact with a rooster infected six months previously developed infection after five week's exposure. Although the virus was not isolated, serological tests demonstrated the presence of neutralizing antibodies.

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